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BRIEF COMMUNICATION

Triacontanol-induced changes in the growth, photosynthetic pigments, cell metabolites, flowering and yield of green gram

G. KUMARAVELU, V. DAVID LIVINGSTONE and M.P. RAMANUJAM

Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Pondicherry - 605 008, India

Abstract

Seedlings of green gram [*Vigna radiata* (L.) Wilczek] cultivar KM-2 were sprayed with different concentrations of triacontanol (TRIA) (0, 0.5, 1.0, and 2.0 mg dm^{-3}) at 15 and 25 days after sowing. Foliar spray of 0.5 mg dm^{-3} TRIA significantly promoted the plant height, fresh mass, and contents of chlorophylls, saccharides, starch, soluble proteins, amino acid and phenols. Leaf nitrate content was reduced by 0.5 and 1.0 mg dm^{-3} TRIA with a corresponding increase in nitrate reductase activity. TRIA of 0.5 mg dm^{-3} stimulated the onset of flowering, pod production and retention, but less number of pods and seeds per plant were observed at 2.0 mg dm^{-3} treatment.

Additional key words: biomass, carotenoids, leaf nitrate, nitrate reductase, saccharides, *Vigna radiata*.

Triacontanol (TRIA), a 30-C primary alcohol was reported as a natural component of plants by Chibnall *et al.* (1933) and its potential growth regulating properties were identified by Ries *et al.* (1977). Since then many investigators have shown that TRIA influences photosynthesis, nutrient uptake, enzymatic activity, *etc.*, (Ries *et al.* 1977, Eriksen *et al.* 1981, Hashim and Lundergan 1985, Miniraj and Shanmugavelu 1987, Oritani 1993, Muthuchelian *et al.* 1995, 1996). To boost yield of several crops complete information is still needed to standardise concentrations, timing, and rate of application of this growth regulator.

Seeds of green gram [*Vigna radiata* (L.) Wilczek] cultivar KM-2 were grown in earthenware pots in greenhouse (day/night temperature 36 ± 2/18 ± 2 °C, relative humidity 60 ± 5 %, maximum irradiance (PAR) 1400 μmol m^{-2} s^{-1}, photoperiod 12 - 14 h). The seedlings (10 days after sowing DAS) were inoculated with 200 mg of commercial preparation of *Rhizobium* inoculum. TRIA solutions of different concentrations (0.5, 1.0, and 2.0 mg dm^{-3}) with the addition of Tween-20 (0.1 % m/v) as surfactant were sprayed at 15 and 25 DAS. Glass distilled water was used for controls. After 5 d, the plant parts were weighed in a digital balance (*Anamed*, Mumbai, India). For dry mass measurements, they were dried in an oven at 80 °C for 48 h. From the 40th day onwards, the number of flowers and pods were counted and weighed at regular intervals of 3 d. For biochemical estimations, the first trifoliate leaves (TF1) were used. Chlorophyll *a+b* (Chl) was estimated according of Shoaf and Lium (1976): the fresh leaves were extracted in dimethyl sulphoxide (DMSO) at 60 °C in dark for 1 h and the absorbance was measured at 645 nm (Chl *b*) and 663 nm (Chl *a*) with DMSO blank. Carotenoids (Car) were determined following Ikan (1969); absorbance was read at 480 nm. From the dried leaf tissues, the contents of total soluble sugars and starch were estimated according to Dubois *et al.* (1956) and McCready *et al.* (1950), respectively. The total soluble protein and amino acid contents were estimated using method of Lowry *et al.* (1951) and Moore and Stein (1954), respectively. The orthodihydric (OD) and total phenol contents were assessed following the method of Johnson and Shoal (1952) and Bray and Thorpe (1954), respectively. *In vivo* nitrate reductase was assayed in fresh leaves by the method of Jaworski (1971) with suitable modifications (Muthuchelian *et al.* 1993). Leaf nitrate contents were estimated according to Wooley

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Abbreviations: Car - carotenoids; Chl - chlorophyll; DAS - days after sowing; NRA - nitrate reductase; OD – orthodihydric phenols; TRIA - triacontanol.

E-mail: mali@md3.vsnl.net.in

Table 1. Effect of 0.5, 1.0 or 2.0 mg dm^{-3} TRIA on the shoot and root lengths [cm], fresh masses [g $plant^{-1}$], contents of chlorophylls, carotenoids and proteins [mg g^{-1}(f.m.)], NR activity [nmol(NO_2) kg^{-1}(f.m) s^{-1}], and nitrate, sugars, starch, amino acids, O.D. and total phenol contents [mg g^{-1}(d.m.)]. Values in a row followed by different letters are significantly different according to Tukey's HSD Multiple Range Test at $P = 0.05$, $n = 10$.

Parameter	20 DAS				30 DAS			
	control	0.5	1.0	2.0	control	0.5	1.0	2.0
Root length	9.13a	10.47b	11.35b	9.60a	12.35a	14.09b	13.55b	11.31a
Shoot length	12.40a	12.63a	11.75a	11.40a	13.00a	14.55b	13.12a	12.50a
Plant height	21.53a	23.10b	23.10b	21.00a	25.35a	28.64b	26.67a	23.81a
Root f.m.	0.19a	0.23b	0.21a	0.20a	0.31a	0.39b	0.30a	0.29a
Shoot f.m.	0.69a	0.72a	0.63ab	0.61b	1.51a	1.55a	1.45a	1.35b
Plant f.m.	0.88ab	0.95a	0.84b	0.81b	1.82a	1.94a	1.75ab	1.64b
Chl	2.23a	2.60b	2.31a	2.24a	3.16a	3.23a	2.86b	2.80b
Car	0.35a	0.35a	0.35a	0.37a	0.51a	0.47ab	0.41bc	0.39c
NR activity	1460a	1535a	1530a	1220b	1048a	1136a	881b	726c
Nitrate	6.21a	5.20b	5.48b	7.02c	2.47a	3.73b	4.42bc	4.67c
Protein	1.41a	1.77b	1.56c	1.22d	1.38ac	1.58b	1.44ab	1.28c
Total sugars	42.91a	50.44b	38.13c	29.26d	38.99a	46.34b	32.95c	27.90d
Starch	31.95a	42.58b	24.36c	20.21c	34.75a	51.51b	36.95a	33.97a
Amino acids	3.68a	4.03b	3.28a	2.55c	3.65a	5.37b	3.87a	3.59a
O.D phenol	3.92a	4.07a	4.17a	4.49b	3.21a	4.36b	3.63c	3.89c
Total phenol	10.30a	10.81a	10.67a	11.81b	7.47a	12.70b	12.19b	8.78c

Table 2. Seed yield characteristics of green gram cv. KM-2 sprayed with 0.5, 1.0 or 2.0 mg dm^{-3} TRIA. Differences in a row followed by different letters are significantly different at $P = 0.05$, $n = 10$.

	Control	0.5	1.0	2.0
Pod number [$plant^{-1}$]	16.20a	19.57b	15.48a	12.50c
Seed number [pod^{-1}]	9.33a	10.13b	9.20a	8.07c
Seed mass [g pod^{-1}]	0.21a	0.26b	0.20ac	0.18c
Pod mass [g]	0.32a	0.36b	0.30ac	0.26c

et al. (1960). For statistical analysis, the data were analysed by Tukey's Multiple Range Test (TMRT) at 5 % level of significance (Zar 1984).

Foliar spray of 0.5 and 1.0 mg dm^{-3} TRIA favoured root and plant elongation significantly at 20 DAS, whereas at 30 DAS, the root, shoot and plant height were significantly increased only at 0.5 mg dm^{-3} TRIA (Table 1). Enhanced plant height was also reported by Muthuchelian *et al.* (1995, 1996) in *Erythrina* seedlings. 0.5 mg dm^{-3} TRIA increased the root fresh mass more than the shoot fresh mass. This may be due to an increased water absorption (Bittenbender *et al.* 1978) seen as an immediate response to low concentrations of TRIA or due to promotion of gibberellin activities in the dark (Ries 1985). Plant biomass was generally reduced at 1.0 and 2.0 mg dm^{-3} TRIA but the decrease was significant only at 30 DAS (Table 1).

Increased chlorophyll (Chl) content after the foliar spray of 0.5 mg dm^{-3} TRIA at 20 DAS was in line with the results of Muthuchelian *et al.* (1995). Perhaps the synthesis of Chl may be stimulated by low concentrations of TRIA. On the contrary, reduction in Chl content observed at 30 DAS at 1.0 and 2.0 mg(TRIA) dm^{-3} may be due to inhibition of synthesis of chlorophyll or due to increase in the breakdown of pigments or their precursors (Muthuchelian *et al.* 1995). The carotenoid content was not significantly affected at 20 DAS, whereas at 30 DAS, increased dose of TRIA decreased the content of carotenoids.

The trends in protein and amino acid contents almost paralleled the activity of NRA (Table 1). Such a positive correlation between NRA and leaf protein content after TRIA spray was also reported by Muthuchelian *et al.* (1990) in *Pennisetum*. 0.5 mg dm^{-3} TRIA significantly increased the contents of sugar, starch, proteins, amino acids in the leaves (Table 1) in both stages. Increase in the contents of leaf soluble protein, starch, sugars and free amino acids in *Erythrina* (Kim *et al.* 1989), *Oryza* and *Zea* (Thakur and Thakur 1992), and *Acacia* leaves (Muthuchelian *et al.* 1995) were also reported. Enhanced

content of photosynthetic pigments and other photosynthetic parameters (Miniraj and Shanmugavelu 1987) may be the reasons for the increased biomass, and saccharide and starch contents. The O.D. and total phenol contents were higher in both the stages compared to their respective controls (Table 1). The O.D. phenols are known to increase whenever a stress is imposed on the plant (Deverall 1976).

TRIA (0.5 mg dm^{-3}) speeded up the flowering and also improved the yield. Similar results were reported by Hashim and Lundergan (1985) in strawberry exposed to foliar spray of TRIA (0.1 - 5.0 mg dm^{-3}).

Significant increases in pod number, seed number and mass per plant, seed number and mass per pod were obvious in plants exposed to 0.5 mg dm^{-3} TRIA (Table 2). Improved yield by TRIA application of several important food crops was shown by many workers (Ries *et al.* 1978, Ries 1985, Kawashima *et al.* 1987). Increased uptake of nutrients, enhanced photosynthesis and improved translocation of photosynthates and other metabolites to the reproductive parts (Miniraj and Shanmugavelu 1987) may have contributed to the production of more pods. Yield reduction by TRIA (2.0 mg dm^{-3}) was due to reduced pod number and mass or seed number and mass per pod.

The present investigation indicates that 0.5 mg dm^{-3} TRIA would be beneficial for the green gram crop.

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